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cont

recovering the GKDM from the aqueous medium.

REMARKS

Claims 1-3 have been amended in order to recite the present invention with the specificity required by statute. The subject matter of the amendment may be found in the specification as filed, inter alia, at page 19, lines 10-22. Accordingly, no new matter has been added.

The Examiner has requested that Applicants affirm their provisional election to prosecute invention of: Group I (Claims 1-5, 8-17, 20 and 23-32), drawn to methods of producing GDP, classified in class 435, subclass 105.

In support of the Restriction Requirement, the Examiner notes that Group I is a method of producing GDP-fucose which does not involve recombinant DNA, whereas Group II does involve recombinant DNA. Accordingly, since "one could produce GDP[-fucose] without using transformants", the methods are said to be patentably distinct. Respectfully submitted, the Examiner has mischaracterized Group I. The claims of Group I do not recite a method which excludes use of transformants; rather, they recite a method which is generic to use of transformants. Accordingly, any proper search of Group I necessarily encompasses the subject matter of Group II.

Put another way, if the subject matter of Claim 6 (Group II) was found in the prior art, the subject matter of antecedent claim 1 (Group I) is necessarily unpatentable as well. Since Group II is a related species under Group I (see MPEP §806.04(b)), any restriction requirement must be made under MPEP §806.05 - §806.05(i) and the Examiner has, respectfully submitted, made no such showing. For this reason at least, restriction between these groups is improper.

Nevertheless, to reduce the issues, Applicants' previous oral Restriction Election is hereby affirmed. Rejoinder of withdrawn dependent claims 6, 7, 18, 19, 21, 22, 33 and 34, at least upon the allowance of their elected antecedent claims is respectfully requested.

Claims 1-4, 8-10, 13, 20, 23-25 and 28 are rejected under 35 U.S.C. §102(b) as being anticipated by Yamamoto et al. (Agric. Biol. Chem. 48(3) 823-4 (1984)). Additionally, Claims 1-5, 8-17, 20 and 23-32 are rejected under 35 U.S.C. §103(a) as being unpatentable over Yamamoto in view of Sturla et al., (FEBS Letters 412 (1997) 126-30). This rejection is respectfully traversed in view of the foregoing amendment and the following remarks.

The Rejection Under 35 U.S.C. §102

In support of the rejection over Yamamoto, the Examiner states that the reference discloses a process for producing guanosine 5'-diphospho (GDP)-fucose from GDP 4-keto-6-deoxymannose using E.coli. In Yamamoto, GDP-mannose produced from GMP-Na and glucose as substrates using air-dried yeast cells as an enzyme source is purified, and GDP-fucose is produced using as substrate the purified GDP-mannose, using NADPH as coenzyme and a crude broken cell extract as an enzyme source. Yamamoto discloses that an enzyme system biosynthesizing GDP-fucose from GKDM exists in mammals, plants and certain bacteria.

The process of Yamamoto includes three steps: i) a step of producing GDP-mannose using an air-dried cells of baker's yeast as an enzyme source, and GMP and glucose as substrates, ii) a step of purifying the GDP-mannose, and iii) a step of producing GDP-fucose using, as an enzyme source, a crude purified enzyme fraction of Aerobacter aerogenes or Agrobacterium radiobacter, and using the purified GDP-mannose obtained in

the above ii) as a substrate, and NADPH as a coenzyme. The present invention, as recited in the pending claims, patentably distinguishes Yamamoto as discussed below.

By way of background, Claims 1 and 2 are processes of producing guanosine 5'-diphospho-fucose (GDP-fucose). Claim 1 utilizes aqueous media containing guanosine 5'-diphospho-4-keto-6-deoxymannose (GKDM) and an enzyme source capable of converting GKDM into GDP-fucose. Claim 2 utilize aqueous media containing a guanosine 5'-triphosphate (GTP) precursor, an enzyme source capable of forming GTP from a GTP precursor, and an enzyme source capable of forming GKDM from a saccharide and GTP. Claim 3 is a process of producing guanosine 5'-diphospho-4-keto-6-deoxymannose utilizing aqeous media containing a GTP precursor, an enzyme source capable of forming GTP from a GTP precursor, and an enzyme source capable of forming GKDM from a saccharide and GTP.

The salient differences between the present invention and the prior art include the following.

As disclosed at specification page 2, lines 5-10, GDP-mannose 4,6-dehydrogenase (GMD) which is an enzyme for producing GKDM from GDP-mannose is inhibited by the final product, GDP-fucose. Accordingly, large amounts of GDP-fucose cannot be obtained by enzymatic production using as a substrate a biosynthesis intermediate of GDP-fucose such as GDP-mannose (which is an upstream compound of GKDM), as in Yamamoto.

On the other hand, according to claim 1, inhibition of GMD by GDP-fucose is avoided by using GKDM as a substrate so that GDP-fucose can be effectively produced in large amounts. This result is neither disclosed nor suggested by Yamamoto. The present inventors have found for the first time that GKDM present in a culture broth permeates a cell membrane of a microorganism and is converted into GDP-fucose to

thereby accumulate GDP-fucose in the culture broth, as plainly recited in amended claim 1. Yamamoto neither discloses nor suggests this salient feature of the present invention

As discussed above, claim 1 relates to a process for producing GDP-fucose in which GKDM, an intermediate in biosynthetic pathway of GDP-fucose, is used as a substrate, and a microorganism is used as an enzyme source. Claim 2 relates to a process for producing GDP-fucose in which GKDM is once formed and accumulated using microorganisms as enzyme sources, the GKDM is used as a substrate and a microorganism is used as an enzyme course. Claim 3 relates to a process for producing GKDM using microorganisms as enzyme sources.

Yamamoto discloses a process for producing GDP-fucose using a purified GDP-mannose as a substrate. However, Yamamoto does not disclose a process for producing GKDM in which GKDM is accumulated in an aqueous medium using a GTP precursor (GMP) and a saccharide (glucose) as substrates. As discussed above, GKDM is a biosynthesis intermediate of GDP-fucose, and is considered to be converted into GDP-fucose immediately after the production of GKDM. The present inventors have found for the first time that unstable GKDM can be formed and accumulated in an aqueous medium by using a microorganism capable of producing GKDM from a GTP precursor and a saccharide in such a large amount that GKDM can be used as a substrate in a later step for producing GDP-fucose. Such process is simply not disclosed by Yamamoto.

Yamamoto neither discloses nor suggests a process for producing GDP-fucose in which GKDM is once formed and accumulated in an aqueous medium by using, as an enzyme source, a culture broth of a microorganism capable of producing GKDM from a GTP precursor (GMP) and a saccharide (glucose) or a treated product of the culture broth, and as substrates, a GTP precursor (GM) and a saccharide (glucose), and then GDP-fucose is produced in a large amount from the GKDM in the aqueous medium

using, as an enzyme source, a culture broth of a microorganism capable of producing GDP-fucose from GKDM or a treated product of the culture broth, as recited in claim 2 of the present application.

As discussed above, an enzyme, GMD, which converts GDP-mannose to GKDM is inhibited by GDP-fucose. However, according to the process recited in claim 2, GKDM is once accumulated in an aqueous medium, and then GKDM is converted into GDP-fucose. Therefore, production inhibition of GDP-fucose caused by inhibition of GMP by GDP-fucose is avoided so that GDP-fucose can be produced in a large amount.

In Example 5 of the present specification, comparison of GDP-fucose productivity was carried out between the process according to the present application and the process for directly producing GDP-fucose from a compound which becomes a substrate of GDP-fucose which is an upstream compound of GKDM, such as GDP-mannose, without once accumulating GKDM. The results shows that the productivity of the former process is about 4 times as high as that of the latter process. This high productivity is obtained by avoiding end product inhibition of GMD by once forming and accumulating GKDM and then converting the GKDM into GDP-fucose. This improvement entirely unexpected in view of the prior art, and is certainly useful to those of ordinary skill.

The Rejection Under 35 U.S.C. §103

As understood, the Examiner asserts that inhibition of GKDM by GDP-fucose can be avoided by using E. coli having strong GMD and WcaG activity. However, organisms originally have a mechanism of inhibition by a final product (feedback inhibition) so that a biosynthesis enzyme relating to synthesis of a final product is inhibited by the final product to thereby control excessive substances. Therefore, even if

the GMD and WcaG activity is strengthened, the feedback inhibition per se by the final product cannot be avoided.

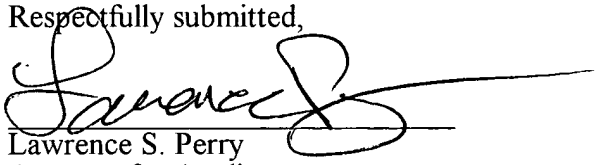
In Example 5 of the present specification, productivity of GDP-fucose was carried out between the process according to the present application was compared to between a process obtained by combination of Yamamoto and Sturla, that is, the process in which a process for directly producing GDP-fucose using, as enzyme sources, E. coli having high GMD activity and E. coli having WcaG activity, and using, as substrates, a GTP precursor (GMP) and a saccharide (glucose) without once accumulating GKDM. As discussed earlier, the productivity according to the present application is about 4 times higher than that according to the process obtained by combination of Yamamoto and Sturla. Accordingly, it is apparent that GDP-fucose cannot be efficiently produced in a large amount by only using E. coli having strong GMD and WcaG activity.

In view of the above amendments and remarks, Applicants submit that all of the Examiner's concerns are now overcome and the claims are now in allowable condition. Accordingly, reconsideration and allowance of this application is earnestly solicited.

Claims 1-3, 5-7, 9-22 and 24-34 remain presented for continued prosecution, claims 6, 7, 18, 19, 21, 22, 33 and 34 being withdrawn from prosecution and rejoinder thereof being respectfully requested.

Applicants' undersigned attorney may be reached in our New York office by telephone at (212) 218-2100. All correspondence should be directed to our below listed address.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE TO CLAIMS

1. (Amended) A process for producing guanosine 5'-diphospho-fucose, comprising:

allowing guanosine 5'-diphospho-4-keto-6-deoxymannose ("GKDM") and an enzyme source to be present in an aqueous medium, wherein the enzyme source is a culture broth of a microorganism capable of converting GKDM into guanosine 5'-diphospho-fucose ("GDP-fucose") or a treated product of the culture broth selected from the group consisting of a concentrated product of the culture broth, a dried product of the culture broth, cells obtained by centrifuging the culture broth, a dried product of the cells, a freeze-dried product of the cells, a surfactant-treated product of the cells, a solvent-treated product of the cells, an enzyme-treated product of the cells and an immobilized product of the cells;

forming and accumulating GDP-fucose in the aqueous medium; and

recovering the GDP-fucose from the aqueous medium.

2. (Amended) A process for producing guanosine 5'-diphospho-fucose, comprising:

allowing a guanosine 5'-triphosphate ("GTP") precursor, a saccharide and enzyme sources to be present in an aqueous medium, wherein the enzyme sources are (i) a culture broth of a microorganism capable of forming GTP from [a] said GTP precursor or a treated product of the culture broth, and (ii) a culture broth of a

microorganism capable of forming guanosine 5'-diphospho-4-keto-6-deoxymannose ("GKDM") from [a] said saccharide and GTP or a treated product of the culture broth;

forming and accumulating GKDM in the aqueous medium;

converting the accumulated GKDM into guanosine 5'-diphospho-fucose ("GDP-fucose") using, as an enzyme source, a culture broth of a microorganism capable of converting GKDM into GDP-fucose or a treated product of the culture broth to form and accumulate GDP-fucose in the aqueous medium; and

recovering the GDP-fucose from the aqueous medium,

wherein the treated products of the culture broth are treated products independently selected from the group consisting of a concentrated product of the culture broth, a dried product of the culture broth, cells obtained by centrifuging the culture broth, a dried product of the cells, a freeze-dried product of the cells, a surfactant-treated product of the cells, a solvent treated product of the cells, an enzyme-treated product of the cells and an immobilized product of the cells.

3. (Amended) A process for producing guanosine 5'-diphospho-4-keto-6-deoxymannose, comprising:

allowing a guanosine 5'-triphosphate ("GTP") precursor, a saccharide and enzyme sources to be present in an aqueous medium, wherein the enzyme sources are (i) a culture broth of a microorganism capable of forming GTP from [a] said GTP precursor or a treated product of the culture broth, and a (ii) culture broth of a microorganism capable of forming guanosine 5'-diphospho-4-keto-6-deoxymannose ("GKDM") from [a] said saccharide and GTP or a treated product of the culture broth,

wherein the treated product of the culture broth is a treated product

independently selected from the group consisting of a concentrated product of the culture broth, a dried product of the culture broth, cells obtained by centrifuging the culture broth, a dried product of the cells, a freeze-dried product of the cells, a surfactant-treated product of the cells, a solvent- treated product of the cells, an enzyme-treated product of the cells and an immobilized product of the cells,

forming and accumulating GKDM in the aqueous medium; and

recovering the GKDM from the aqueous medium.